Differential Reaction of Tomato Chromosomes with Hydroxylamine

A number of chemicals which produce localized breakage in the chromosomes have been known since the early work of McLEISH[1] and others[2]. In more recent years, following a better understanding of the specificity of action of some of these chemicals (FREESE[3]), attempts have been made to correlate the induced chromosomal damage with the distribution of deoxyribonucleic acid bases in the affected regions. Thus, SOMERS and HSU[4] have concluded, on the basis of treatment with hydroxylamine, that certain segments of the chromosomes in Chinese hamster cells may be relatively rich in G-C (guanine-cytosine) base pairs. It had been shown earlier by FREESE et al.[5] and by SCHUSTER[6] that the reactivity of this compound with cytosine at a particular pH is significantly greater than with other DNA bases which hardly show any reaction.

We have been studying the effect of this chemical on the chromosomes of tomato. The treatment is given by keeping cut floral shoots of the variety 'Improved Meeruti' in freshly prepared 0.01 *M* hydroxylamine (NH_2OH, HCl) solution for a period of 12 h at a temperature of 25 ± 1°C, followed by 36 h in distilled water. The solution was prepared in a phosphate buffer, adjusting the pH finally to 6.8 with the addition of a few drops of NaOH. In the corresponding control series, the cut shoots were kept in distilled water for the entire duration of 48 h.

Observations on pollen mother cells from the treated shoots showed a rather drastic effect of the chemical on chromosomal structure. In the control series, the chromosomes form 12 bivalents all of which could be clearly seen (Figure 1). In contrast to this, in many of the treated pollen mother cells the chromosomes appeared disorganized and degenerated, so much so that the full complement could not be stained in many of the nuclei. Thus, 30% of the cells were found to show fewer than 12 bivalents. The stainable chromosomes, with their partially disorganized structure, tended to clump together. That all the chromosomes were not affected to the same degree was further indicated by observations on another group of 22% cells in which 1–3 of the chromosome pairs appeared much less disorganized than others, the latter showing poor stainability both with Feulgen and carmine stain (Figure 2). In both these groups of cells chromosome 2, which can be recognized by its association with the nucleolus[7,8], was

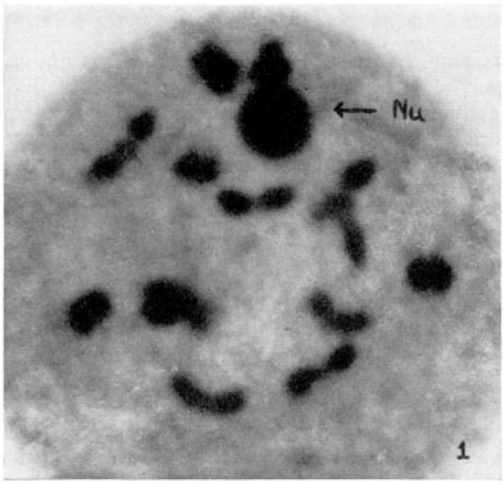

Fig. 1. PMC from the control material showing the 12 bivalents and the single nucleolus (marked Nu). One of the chromosome pairs (chromosome 2) is associated with the nucleolus. × 3000. (Acetocarmine squash).

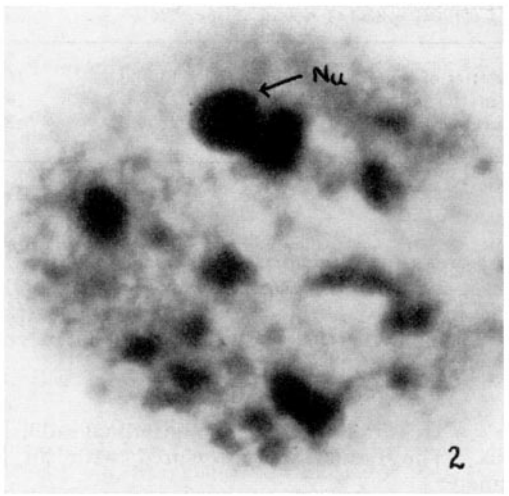

Fig. 2. PMC from a treated shoot showing disorganization of chromosomal structure. Three of the bivalents, including one associated with the nucleolus, indicate better structure and stainability. × 2600. (Acetocarmine.)

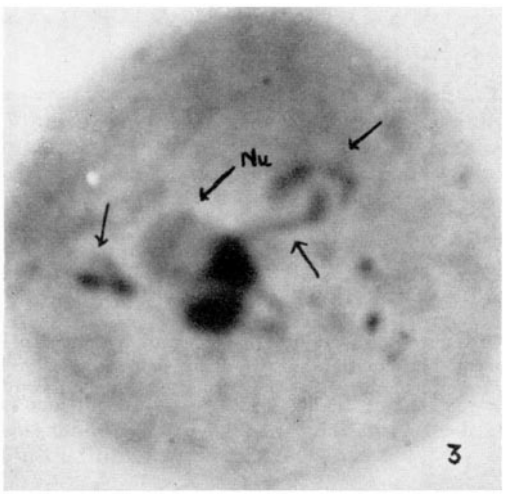

Fig. 3. Another cell from the treatment series in which only the bivalent associated with the nucleolus and one other pair appear well stained. The other chromosomes, except for some weakly-stained thread-like structures (arrow), indicate complete degeneration. × 2600. (Feulgen squash.)

[1] J. McLEISH, Heredity *8*, 385 (1954).
[2] B. KIHLMAN and A. LEVAN, Hereditas *37*, 382 (1951).
[3] E. FREESE, in *Molecular Genetics*, Part I (Ed.: J. H. TAYLOR; Academic Press, New York 1963), p. 207.
[4] E. C. SOMERS and T. C. HSU, Proc. Nat. Acad. Sci. US *48*, 937 (1962).
[5] E. FREESE, E. BAUTZ-FREESE, and E. BAUTZ, J. mol. Biol. *3*, 133 (1961).
[6] H. SCHUSTER, J. mol. Biol. *3*, 447 (1961).
[7] D. W. BARTON, Am. J. Bot. *37*, 639 (1950).
[8] W. GOTTSCHALK, Chromosoma *4*, 298 (1951).

one of those relatively less affected by the treatment. This differential reaction was more clearly observed in another group of 8% cells in which chromosome 2 alone, or together with one other bivalent, could be seen with Feulgen staining, although in a disorganized state, while all other pairs were so completely degenerated that, except for a few small thread-like fragments, they could not be stained at all (Figure 3). The rest of the pollen mother cells were relatively free from nuclear damage, although many of them showed such irregularities as failure of pairing or differential condensation of their chromosomes.

In general, chromosome 2, and some others which could not be identified, were found to survive the treatment better than others.

The drastic effect of hydroxylamine on tomato chromosomes can probably be attributed to the high concentration used in the course of the present study (SOMERS and HSU[4]). The differential response of some of the chromosomes may possibly point to inhomogeneity in the concentration of G-C base pairs in the different members of the complement. Alternatively, the different chromosomes may be so organized in their structure that not all of them permit an equally free reaction with the chemical. It is proposed to obtain further evidence in relation to possible concentration differences of the bases by studying the incorporation of their tritium labelled precursors in the different chromosomes, although the unfavourable nature of mitotic preparations in tomato makes this difficult.

The interchromosome base composition differences, if proved, may have interesting genetical implications. The distribution of the known genes in the tomato complement is known to be highly asymmetrical; a number of chromosomes, particularly chromosome 2, have a disproportionately and unaccountably large number of genes located on them[9]. A further discussion of this aspect, however, is not considered advisable at this stage in the absence of more definite evidence[10].

Zusammenfassung. Hydroxylamin, welches spezifisch mit Cytosin reagiert, stört die Struktur der verschiedenen Chromosomen in Pollenmutterzellen der Tomate unterschiedlich stark. Das Chromosomenpaar Nr. 2 wird wesentlich weniger stark geschädigt als die übrigen. Die Bedeutung dieses Befundes wird kurz angedeutet.

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[9] C. M. RICK, Proc. Nat. Acad. Sci. US *45*, 1515 (1959).

[10] We thank Dr. B. P. PAL and Dr. M. S. SWAMINATHAN for their interest and encouragement.

Halbe haploide Chromosomenzahl im Hoden von *Myrmica sulcinodis* Nyl. (Formicidae)

Bei allen bisher untersuchten Formiciden zeigten die Männchen die haploide Chromosomenzahl gegenüber den Weibchen (SMITH und PEACOCK[1], HAUSCHTECK[2–4]). Ältere, hier nicht zitierte Arbeiten sind bei HAUSCHTECK[2,3] besprochen.

Myrmica sulcinodis wurde im Inntal bei Ramosch gesammelt. Aus Kopfganglien und Gonaden von Vorpuppen beider Geschlechter wurden Quetschpräparate hergestellt.

In Ovarien von Weibchen kamen Chromosomenzahlen zwischen 48 und 57 vor. Zwischen diesen Grenzen muss also die diploide Zahl liegen. Sie ist mit 56 anzunehmen, da in zwei Männchen 28 Chromosomen im Hoden einwandfrei zu ermitteln waren.

Die genaue Untersuchung der Hoden dieser beiden Männchen ergab einen unerwarteten Befund: Viele Kerne zeigten eine Chromosomenzahl, die noch um die Hälfte niedriger war als die haploide Zahl 28. Zusätzlich wurden diploide Kerne gefunden. Die Häufigkeiten der einzelnen Kerntypen sind in Tabelle I wiedergegeben.

Die haploiden Kerne mit 28 Chromosomen stellen den erwarteten Typ dar. Die Zahl solcher Kerne in den beiden Männchen ist jedoch viel zu gering, um für die Spermienproduktion bedeutsam zu sein. Die diploiden, vermutlich nicht generativen Kerne sind bei Ameisen im Hoden keine Seltenheit. Ihre Bedeutung wurde nicht verfolgt. Die grosse Masse der Kerne enthält 14 Chromosomen. Die naheliegende Bezeichnung «n/2» für diesen Chromosomensatz darf möglicherweise nicht benutzt werden, denn bisher ist nicht bekannt, ob alle Chromosomensätze mit 14 Chromosomen einander homolog sind. Falls ein Beweis für die Homologie erbracht werden kann, wäre der Chromosomensatz der Weibchen nicht diploid sondern tetraploid. Das vorliegende Material gibt keinen Aufschluss darüber, ob aus diesen «n/2»-Kernen funktionstüchtige Spermien gebildet werden. Es wäre immerhin möglich, dass beide Geschlechter parthenogenetisch entstehen.

Die Figur zeigt zwei Kerne desselben Hodens. Dem cytologischen Bild ist nicht sicher zu entnehmen, ob es

Tabelle I. Häufigkeiten verschiedener Chromosomenzahlen in den Hoden zweier Männchen von *Myrmica sulcinodis*. Beim Auszählen der Häufigkeiten wurden auch solche Kerne berücksichtigt, deren Chromosomenzahl nur geschätzt werden konnte.

♂ No.	Anzahl der Kerne mit den Chromosomenzahlen		
	«n/2» = 14	n = 28	2n = 56
1	210	27	11
2	390	8	8

[1] I. C. SMITH und A. D. PEACOCK, Proc. Roy. Soc., Edinb. *B 66*, 235 (1957).

[2] ELISABETH HAUSCHTECK, Rev. Suisse Zool. *68*, 218 (1961).

[3] ELISABETH HAUSCHTECK, Vjschr. Naturforsch. Ges. Zürich *107*, 213 (1962).

[4] ELISABETH HAUSCHTECK, Proc. 11, Intern. Congr. Genetics (1963).